Research Article

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Pharmacogenomics

Transient receptor potential polymorphism and haplotype associate with crisis pain in sickle cell disease

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Aim: Episodes of acute pain crisis contribute to considerable morbidity and mortality in sickle cell disease (SCD). Incomprehensive understanding of the underlying pain heterogeneity results in inadequate pain management. The transient receptor potential (TRP) family of voltage-gated ion channels acts as sensory transducers of diverse noxious stimuli. We performed an association study of polymorphisms in candidate genes *TRPV1* and *TRPA1* with pain in SCD patients. **Methods:** Utilization rate, in other words, number of emergency department/acute care center admissions over 12 months as a result of pain crisis, served as a marker for acute pain. **Results & conclusion:** We identified that rs920829 (incident rate ratio = 1.44, p = 0.027 additive; IRR=1.68, p=0.008 recessive models of negative binomial regression) and the CGAGG haplotype of *TRPA1* (odds ratio = 0.218, p = 0.009) were significantly associated with utilization rate, suggesting that *TRPA1* gene polymorphisms may influence acute pain crisis in SCD.

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Keywords: pain crisis • polymorphism • transient receptor potential • TRPV1 • TRPA1

Episodes of acute pain, also known as vaso-occlusive painful crises, lead to considerable morbidity, mortality and healthcare cost in patients with sickle cell disease (SCD) [1–4]. Chronic pain is also prevalent in SCD, although it is less well understood and studied [3,5,6]. Both acute and chronic pain contribute to overall pain severity. Another aspect of pain in SCD is its high heterogeneity that is well recognized by patients and clinicians, but only recently has been reported. For example, one study showed that 29% of patients had no episode of acute pain or healthcare utilization per year, whereas 16.9% had three or more [7]. In another study conducted during routine outpatient clinic visits, 35% of patients reported no pain, while 19% reported severe pain [3]. To understand pain variations, we take a molecular genetic approach to examine candidate genes and mechanisms that may contribute to SCD pain.

The transient receptor potential (TRP) family of voltage-gated ion channels acts as the primary sensory transducers of diverse noxious stimuli and mediate pain sensitivity [8]. Comprised of six related protein families identified by their homology, TRP channels have six-transmembrane polypeptide subunits that assemble as tetramers to form pores permeable to cations. TRP channels and their splice variants are ubiquitously expressed. TRPV1 and TRPA1 subfamilies have been extensively studied and are implicated in playing essential roles in pain transduction and sensitization. TRPV1 is a Ca^{2+}-permeable cationic channel identified by the 'hot' pepper-derived vanilloid compound, capsaicin, as a ligand [9,10]. TRPV1 mediates the response to noxious heat (>43°C) and protons, and its signal is inhibited by intracellular phosphatidylinositol-4,5-bisphosphate C (PIP_2) [9,11]. TRPA1, also known as

ANKTM1, is also a Ca^{2+}-permeable ion channel and is potentiated by noxious cold (<17°C), mechanical stimuli and noxious environmental chemicals [12–15].

Growing evidence implicates an important role for TRPA1 and TRPV1 in the generation and/or maintenance of inflammatory and neuropathic pain, demonstrated not only in preclinical models but also in human experimental pain models and pain patients [16]. Chemical inhibition of TRPV1, but not TRPA1, attenuated mechanical behavioral hypersensitivity in Berkeley sickle cell transgenic mice [17]. Despite the accumulating evidence for TRP channels' essential role in transducing and mediating pain, supported by *in vivo* physiological and pharmacological data from animal models and clinical data from patients with pain, there is limited knowledge of the influence of genetic polymorphisms of TRP channels on pain. Several SNPs of *TRPA1* and *TRPV1* have shown association with various pain phenotypes in recent clinical reports. *TRPA1* 710G>A rs920829 was associated with abnormal heat sensation and *TRPV1* 1911A>G rs8065080 was associated with cold hypoalgesia in neuropathic pain patients [18]. *TRPV1* 315G>C rs222747 showed lower susceptibility of painful functional dyspepsia [19]. *TRPV1* 2841C>T rs222741 was associated with higher risk of migraine [20]. Although genetic mechanism of SCD is well studied, the genetic basis of pain heterogeneity in SCD patients is poorly understood. There is no study that has been carried out to examine the influence of the genetic variance of TRP channels on pain in SCD patients. In this study, we performed analysis of eight *TRPA1* and three *TRPV1* SNPs in SCD patients regarding utilization and Composite Pain Index (CPI), as markers for acute and chronic pain in SCD.

Methods

Subjects

The study was approved by the University of Illinois (IL, USA) at Chicago Institutional Review Board. Study participants gave written informed consent and blood and/or buccal swab samples were collected at the University of Illinois (UI) Hospital and Health Sciences System for DNA extraction. Patient eligibility criteria are described in detail in another article [21]. Analysis was conducted on subjects with SCD where both clinical data and genetic samples were available. Due to the exploratory nature of this study, a power analysis was not performed.

Acute pain assessment

In our study, the number of admissions to the emergency department and/or acute care center as a result of pain crisis was counted as utilization and serves as the surrogate marker for acute pain. Utilizations resulted from sickle cell pain crises for the subsequent 12 months after the patient completed the baseline pain assessment were collected by medical record review for UI utilization or biweekly telephones calls for non-UI utilization. Utilization groups were categorized by 0 events (zero), 1–3 events (low) or 4–38 events (high) based on a previous study of SCD adults where CPI score predicted the subsequent 1-year acute care utilization by these three groups [21].

Chronic pain assessment

Subjects self-administered the pain assessment tool, PAIN *Report* It®, an electronic touch screen format of the 1970 version of McGill Pain Questionnaire [22] that records pain location, intensity, quality and pattern [3,21,23]. A single CPI value with a range of 0–100 is calculated to represent the multidimensional pain experience [21]. The number of pain sites; average of current, least and worst pain intensity in the past 24 h; pain rating index total; and a pain pattern score that ranges from 0 to 6 were each converted to a 0–100 scale, then subsequently averaged [22,24,25]. This tool and its measure have previously been tested for patients with SCD [21].

DNA extraction & genotyping

DNA extraction from blood samples was done by the QuickGene-mini80 isolation device with the QuickGene DNA whole blood extraction method (AutoGen, MA, USA) and a modified salting out procedure [26]. A modified phenol/chloroform procedure [27] was used to extract DNA from buccal samples. The MassARRAY iPLEX Platform (Sequenom, CA, USA) [28] was used to genotype all SNPs except for rs224534 and rs222747. PCR-RFLP was performed for these two SNPs according to previously published methods [29]. The genotyping success rate was more than 90%.

Statistical analysis

A χ^2 goodness-of-fit test was used to calculate Hardy–Weinberg equilibrium. SNP influence on utilization was analyzed by an additive, dominant and recessive negative binomial regression model [30,31] adjusted for age, sex

Table 1. Summary of demographics and phenotype data.Items

Subject demographics	Items	Values
Age	Mean ± SD	34.2 ± 11.8
	Minimum	15
	Maximum	70
Sex, n (%)	Female	86 (65.2)
	Male	46 (34.8)
Sickle cell type†, n (%)	SCD-SS	102 (77.3)
	SCD-SC	15 (11.4)
	Others	15 (11.4)
Phenotype summary		
Utilization (number of events)	Mean ± SD	4.5 ± 5.3
	Minimum	0
	Maximum	38
Utilization groups, n (%)	Zero (0)	19 (14.4)
	Low (1–3)	57 (43.2)
	High (4–38)	56 (42.4)
Composite pain index	Mean ± SD	40.7 ± 13.4
	Minimum	14.8
	Maximum	86.5

†Sickle cell types: SCD-SS, SCD-SC; others include SCD-sickle β+ thalassemia, SCD-sickle β0 thalassemia and SCD-sickle α thalassemia.
SCD: Sickle cell disease; SCD-SC: SCD-sickle hemoglobin C; SD: Standard deviation; SCD-SS: SCD-homozygous hemoglobin S, sickle cell anemia.

and sickle cell type. SNP effects on three different utilization groups were analyzed by an additive, dominant and recessive ordinal logistic regression model adjusted for the same covariates [21]. The effect of SNPs on CPI value was analyzed by an additive, dominant and recessive multiple linear regression model [32,33] adjusted for age, sex and sickle cell type. Major alleles are reference in all analyses. The dominant model for the following SNPs was not applicable due to low minor allele frequency: rs8065080 and rs13255063. *TRPV1* rs224534-dominant model in the analysis of utilization groups was also eliminated from analysis due to low minor allele frequency. Analysis was performed on Statistical Package for the Social Sciences (SPSS) software (version 20; IBM, NY, USA) and R (version 3.4.0) [34,35]. The linkage disequilibrium (LD) analysis was plotted and performed in Haploview version 4.2 (Broad Institute, MA, USA) [36]. Haplotype association analyses were performed with PLINK (Massachusetts General Hospital, the Broad Institute of Harvard and MIT, MA, USA) [37,38]. Hap-logistic options were used on PLINK to include covariates (age, sex, ethnicity and sickle cell type) for utilization group analyses. Due to the exploratory nature of this study, multiplicity controls were not applied.

Results

Demographics for 132 self-reported African–American subjects are provided in Table 1. This study captured subjects with an age range of 15–70 years with a mean of 34.2 years. More female subjects [65%] participated in this study than males. The sickle cell anemia genotype (SCD-SS) constitutes the majority of sickle cell types in our study [77%]. The number of utilizations within 1 year of taking the pain assessment ranged widely from 0 to 38 events with a mean of 4.5. Utilization was also categorized into three groups (zero, low, high) where zero utilization was experienced by 19 subjects, 1–3 utilizations by 57 subjects and 4–38 utilizations by 56 subjects. CPI mean was recorded to be 40.7 with a wide range of 14.8–86.5.

Genotyping was performed for eight *TRPA1* SNPs and three *TRPV1* SNPs. Genotype and allele frequencies were presented in Table 2, where SNPs are listed in order of chromosome position for the gene according to GenBank GRCh38 (Genome Reference Consortium human reference assembly). None of these SNPs deviated significantly from Hardy–Weinberg equilibrium ($p > 0.05$). All three *TRPV1* SNPs are nonsynonymous, whereas only one *TRPA1* SNP is nonsynonymous. The major allele and minor allele are noted because all analyses were performed with the major allele as the reference.

TRPA1 rs920829 genotypes were statistically significantly associated with utilization (acute pain) in the both the additive and recessive model for negative binomial regression analysis (incident rate ratio = 1.44 [95% CI:

Table 2. Allele and genotype frequencies.

Gene	dbSNP ID	Chromosome position†	Allele, n (%)		Genotypes, n (%)		
			Major	Minor	Major homozygote	Heterozygote	Minor homozygote
TRPA1	1947913	72014779	T, 127 (61)	A, 81 (39)	38 (37)	51 (49)	15 (14)
	13279503	72027391	G, 184 (83)	C, 38 (17)	76 (68)	32 (29)	3 (3)
	13255063	72047300	T, 201 (91)	A, 19 (9)	91 (83)	19 (17)	0 (0)
	1025928	72051023	C, 149 (67)	T, 73 (33)	50 (45)	49 (44)	12 (11)
	3735942	72053738	G, 136 (61)	A, 86 (39)	42 (38)	52 (47)	17 (15)
	3735943	72053767	G, 112 (51)	A, 108 (49)	31 (28)	50 (45)	29 (26)
	920829	72065468	G (Glu), 159 (72)	A (Lys), 61 (28)	55 (50)	49 (45)	6 (5)
	1443952	72068417	G, 141 (64)	A, 81 (36)	44 (40)	53 (48)	14 (13)
TRPV1	8065080	3577153	T (Ile), 193 (88)	C (Val), 27 (12)	83 (75)	27 (25)	0 (0)
	224534	3583408	G (Thr), 243 (92)	A (Ile), 21 (8)	112 (85)	19 (14)	1 (1)
	222747	3589906	C (Ile), 234 (89)	G (Met), 30 (11)	105 (80)	24 (18)	3 (2)

dbSNP IDs are from the National Center for Biotechnology Information (NCBI) database [50].
†Chromosome position is from NCBI GRCh38 assembly. *TRPV1* resides in chromosome 17, *TRPA1* resides in chromosome 8.
Glu: Glutamic acid; Ile: Isoleucine; Lys: Lysine; Met: Methionine; Thr: Threonine; Val: Valine.

1.02–2.04], 1.68 [95% CI: 1.15–2.48]; p = 0.027, 0.008) (Table 3). The AA and AG genotypes showed a 68% increase in the rate of utilization over the major homozygous GG genotype. Also, each A allele contributed to a 44% increase in utilization rate. The dominant model did not yield a significant association (incident rate ratio = 0.82 [95% CI: 0.35–2.11]; p = 0.656). These analyses show an association of *TRPA1* SNPs with utilization counts, however, none of the three nonsynonymous SNPs in *TRPV1* gene showed significant association with utilization in our study (Tables 3 & 4). None of the SNPs in *TRPV1* and *TRPA1* showed statistically significant associations with CPI (Table 5).

Upon reanalyzing our data with four more subjects of Caucasian and Hispanic ethnicity, who had earlier been excluded from the analysis, we observed a trend for decrease in CPI associated with the G allele of *TRPV1* SNP rs222747 in the additive multiple linear regression model (B = -4.67 [95% CI: -9.50–0.15]; p = 0.058). However, dominant and recessive models did not show any trend. In this extended cohort (n = 136), SNP rs920829 still exhibited significant association with the utilization rate in the additive (p = 0.027) and recessive models (p = 0.008).

An LD analysis was further performed for eight *TRPA1* SNPs (Figure 1A) and three *TRPV1* SNPs (Figure 1B) in the extended cohort. *TRPV1* SNPs do not show any significant LD among them. *TRPA1* contains one haplotype block spanning 17 kb with five SNPs (rs1025928–rs3735942–rs3735943–rs920829–rs1443952). A haplotype analysis was performed with these *TRPA1* SNPs in LD as shown in Table 6. A logistic regression was performed for haplotypes and utilization groups where utilization was divided into 0 versus 1 or more and 0–3 versus 4 or more numbers of utilization. A significant association was found for haplotype CGAGG (odds ratio = 0.218, p = 0.009) when analyzed for 0–3 versus 4 or more utilizations. This haplotype contains the rs920829 G allele and rs1025928 C allele. Taken together, these data suggest that *TRPA1* gene polymorphisms and haplotypes may contribute to the heterogeneity of acute pain in SCD.

Discussion

In this study, we evaluated the potential role of the TRP channel gene polymorphisms with the acute pain crisis and chronic pain in SCD patients. We observed an association of *TRPA1* polymorphism and haplotype with acute care utilization due to painful crises in SCD patients. Notably, *TRPA1* rs920829 AA/AG genotypes showed 1.68-times the utilization rate of GG genotype (p = 0.008). Haplotype analyses revealed that the CGAGG haplotype of *TRPA1* containing rs920829 was associated with decreased odds of utilization (p = 0.009), which is consistent with the findings from single SNP analyses with rs920829 and rs1025928. The three *TRPV1* polymorphisms were not found to be associated with either utilization or CPI in our study.

Genetic polymorphisms of TRP channels have been studied in associations with several other neuropathic pain states. Binder *et al.* assessed the role of *TRPA1* and *TRPV1* polymorphisms in modulating the somatosensory function in neuropathic pain patients in a German cohort and found that GA and AA genotypes of *TRPA1*

Table 3. Effects of *TRPV1* and *TRPA1* SNPs on utilization.

Gene	dbSNP ID	Model	IRR (95% CI)†	p-value
TRPA1	1947913	Add	1.02 (0.74–1.41)	0.898
		Dom	0.80 (0.44–1.51)	0.480
		Rec	1.16 (0.75–1.77)	0.509
	13279503	Add	0.95 (0.64–1.43)	0.775
		Dom	0.40 (0.11–1.70)	0.178
		Rec	1.02 (0.66–1.59)	0.928
	13255063	Add	0.91 (0.54–1.58)	0.709
		Dom	N/A‡	N/A
		Rec	0.91 (0.54–1.58)	0.709
	1025928	Add	1.19 (0.87–1.63)	0.253
		Dom	0.90 (0.48–1.78)	0.749
		Rec	1.40 (0.93–2.11)	0.094
	3735942	Add	1.03 (0.77–1.38)	0.843
		Dom	1.23 (0.71–2.20)	0.445
		Rec	0.95 (0.61–1.43)	0.768
	3735943	Add	0.85 (0.64–1.13)	0.238
		Dom	0.77 (0.48–1.24)	0.251
		Rec	0.85 (0.54–1.32)	0.465
	920829	Add	1.44 (1.02–2.04)	0.027
		Dom	0.82 (0.35–2.11)	0.656
		Rec	1.68 (1.15–2.48)	**0.008**
	1443952	Add	1.12 (0.83–1.50)	0.462
		Dom	1.51 (0.85–2.82)	0.148
		Rec	1.00 (0.65–1.52)	0.998
TRPV1	8065080	Add	1.12 (0.71–1.80)	0.618
		Dom	N/A‡	N/A
		Rec	1.12 (0.71–1.80)	0.618
	224534	Add	1.16 (0.75–1.86)	0.552
		Dom	N/A‡	
		Rec	1.17 (0.73–1.95)	0.513
	222747	Add	0.87 (0.60–1.29)	0.485
		Dom	0.91 (0.26–3.78)	0.890
		Rec	0.84 (0.54–1.32)	0.441

†Incident rate ratio and 95% CI.
‡Minor allele frequency is too low for the analysis in a dominant model.
Major alleles are the reference in the analyses.
Regression models are adjusted for age, sex and sickle cell type.
Significant p-values are in bold.
Add: Additive; Dom: Dominant; IRR: Incident rate ratio; N/A: Not applicable; Rec: Recessive.

rs920829 were associated with less paradoxical heat sensation [18]. In another study, the A allele was associated with less paradoxical heat sensation in neuropathic pain patients [39]. In this study, we determined the frequencies of acute pain crisis in SCD by monitoring the number of utilization of emergency department and/or acute care center events. We are conducting a new study with quantitative sensory testing that will determine thermal and mechanical sensitivities in patients with SCD, so it will be interesting to see whether this SNP also influences heat sensation in SCD.

We only found a trend for decreased pain as measured by CPI in subjects with *TRPV1* rs222747 G allele. Subjects with *TRPV1* rs222747 CC genotype were reported to have a lower risk of epigastric pain syndrome in Japanese patients with functional dyspepsia [19]. In phenotypic subpopulations with preserved sensory function, G allele of *TRPV1* rs222747 was found to be associated with cold hypesthesia [18]. In the same study, *TRPV1* rs8065080 G allele was associated with less heat hyperalgesia, less pinprick hyperalgesia and less mechanical hypesthesia, and

Table 4. Effects of *TRPV1* and *TRPA1* SNPs on utilization groups.

Gene	dbSNP ID	Model	Estimate (95% CI)†	p-value
TRPA1	1947913	Add	0.23 (-0.32–0.79)	0.414
		Dom	-0.09 (-1.16–0.99)	0.869
		Rec	0.50 (-0.27–1.27)	0.210
	13279503	Add	-0.44 (-1.10–0.22)	0.198
		Dom	-0.79 (-2.78–1.19)	0.428
		Rec	-0.47 (-1.25–0.30)	0.232
	13255063	Add	-0.32 (-1.23–0.60)	0.495
		Dom	N/A‡	N/A
		Rec	-0.32 (-1.23–0.60)	0.495
	1025928	Add	0.25 (-0.29–0.81)	0.372
		Dom	0.23 (-0.95–1.47)	0.703
		Rec	0.36 (-0.37–1.10)	0.332
	3735942	Add	0.04 (-0.47–0.56)	0.867
		Dom	0.23 (-0.75–1.25)	0.645
		Rec	-0.04 (-0.78–0.70)	0.915
	3735943	Add	-0.25 (-0.75–0.24)	0.317
		Dom	-0.64 (-1.47–0.17)	0.125
		Rec	-0.05 (-0.87–0.76)	0.910
	920829	Add	0.16 (-0.44–0.78)	0.595
		Dom	-0.21 (-1.77–1.39)	0.793
		Rec	0.29 (-0.44–1.03)	0.443
	1443952	Add	0.24 (-0.29–0.78)	0.372
		Dom	0.75 (-0.34–1.91)	0.190
		Rec	0.12 (-0.62–0.86)	0.749
TRPV1	8065080	Add	-0.16 (-0.99–0.68)	0.710
		Dom	N/A‡	N/A
		Rec	-0.16 (-0.99–0.68)	0.710
	224534	Add	0.55 (-0.30–1.47)	0.219
		Dom	N/A‡	N/A
		Rec	0.52 (-0.38–1.47)	0.267
	222747	Add	0.40 (-0.30–1.12)	0.267
		Dom	0.56 (-1.55–2.81)	0.602
		Rec	0.47 (-0.35–1.32)	0.272

†Ordered log-odds estimate and 95% CI.
‡Minor allele frequency is too low for the analysis in a dominant model.
Ordered log-odds estimate that the minor allele would result in a higher utilization group.
Major alleles are the reference in the analyses.
Regression models are adjusted for age, sex and sickle cell type.
Low utilization group (1–3 utilizations), high utilization group (4–38 utilizations).
Add: Additive; Dom: Dominant; N/A: Not applicable; Rec: Recessive.

G allele of *TRPV1* rs222747 with cold hypesthesia in patients with neuropathic pain [18]. In another study to investigate the relative contributions and interactions of gender, ethnicity, psychological and genetic factors on pain sensitivity in humans, it was found that female European–Americans with the *TRPV1* rs8065080 G allele showed less cold sensitivity with longer cold-withdrawal times [40]. We did not see any association of rs8065080 with any pain phenotypes. In a Spanish population-based study, T allele of *TRPV1* rs222741 was associated with higher risk of migraine [20]. *TRPA1* rs11988795 A allele carriers are shown to have less pain tolerance to the cold stimuli and to enhance cold pain perception [41].

TRPV1 and TRPA1 are both Ca^{2+}-permeable channels that respond to different stimuli. TRPV1 is mostly localized on small diameter nociceptive neurons, likely to be unmyelinated C-fibers [42]. Studies using *TRPV1*$^{-/-}$ mice showed that TRPV1 contributed to acute thermal nociception and hyperalgesia after tissue injury [43]. More recently, participation of TRPV1 in transducing and mediating responses to mechanical stimuli has also been

Table 5. Effects of *TRPV1* and *TRPA1* SNPs on composite pain index.

Gene	dbSNP ID	Model	B (95% CI)†	p-value
TRPA1	1947913	Add	3.43 (-0.59–7.45)	0.093
		Dom	4.56 (-3.37–12.50)	0.256
		Rec	4.29 (-1.28–9.86)	0.130
	13279503	Add	-1.86 (-6.79–3.06)	0.455
		Dom	-10.07 (-26.00–5.98)	0.213
		Rec	-1.17 (-6.77–4.43)	0.680
	13255063	Add	2.74 (-4.17–9.65)	0.434
		Dom	N/A‡	N/A
		Rec	2.74 (-4.17–9.65)	0.434
	1025928	Add	-1.18 (-5.06–2.71)	0.549
		Dom	-3.29 (-11.65–5.07)	0.437
		Rec	-0.86 (-6.11–4.40)	0.748
	3735942	Add	1.81 (-1.91–5.54)	0.337
		Dom	1.54 (-5.61–8.70)	0.670
		Rec	2.85 (-2.48–8.18)	0.291
	3735943	Add	0.04 (-3.56–3.64)	0.981
		Dom	-2.50 (-8.45–3.46)	0.408
		Rec	2.52 (-3.33–8.38)	0.394
	920829	Add	1.02 (-3.37–5.41)	0.645
		Dom	7.66 (-3.73–19.05)	0.185
		Rec	-0.14 (-5.42–5.14)	0.957
	1443952	Add	2.43 (-1.42–6.27)	0.214
		Dom	3.26 (-4.50–11.02)	0.407
		Rec	3.05 (-2.24–8.34)	0.255
TRPV1	8065080	Add	0.97 (-5.10–7.04)	0.752
		Dom	N/A‡	N/A
		Rec	0.97 (-5.10–7.04)	0.752
	224534	Add	0.35 (-5.70–6.41)	0.909
		Dom	N/A‡	N/A
		Rec	0.63 (-5.88–7.15)	0.848
	222747	Add	-4.12 (-9.17–0.93)	0.109
		Dom	-6.46 (-22.54–9.62)	0.428
		Rec	-4.70 (-10.57–1.18)	0.116

†Unstandardized regression coefficient and 95% CI.
‡Minor allele frequency is too low for the analysis in a dominant model.
Major alleles are the reference in the analyses.
Regression models are adjusted for age, sex and sickle cell type.
Add: Additive; Dom: Dominant; N/A: Not applicable; Rec: Recessive.

Table 6. *TRPA1* haplotype analysis with utilization groups.

Haplotype	Frequency (%)	0 versus 1 or more utilizations		0–3 versus 4 or more utilizations	
		OR	p-value	OR	p-value
CAAGA	36	1.23	0.635	1.36	0.306
TGGAG	27	0.64	0.326	1.34	0.376
CGAGG	11	0.95	0.922	0.22	**0.009**
TGGGG	6	2.69	0.359	1.48	0.484
CGGGG	17	1.77	0.317	1.23	0.583

SNP order in haplotype (rs1025928–rs3735942–rs3735943–rs920829–rs1443952).
Hap-logistic options were used to include covariates (age, sex, ethnicity and sickle cell type).
OR: Odds ratio.

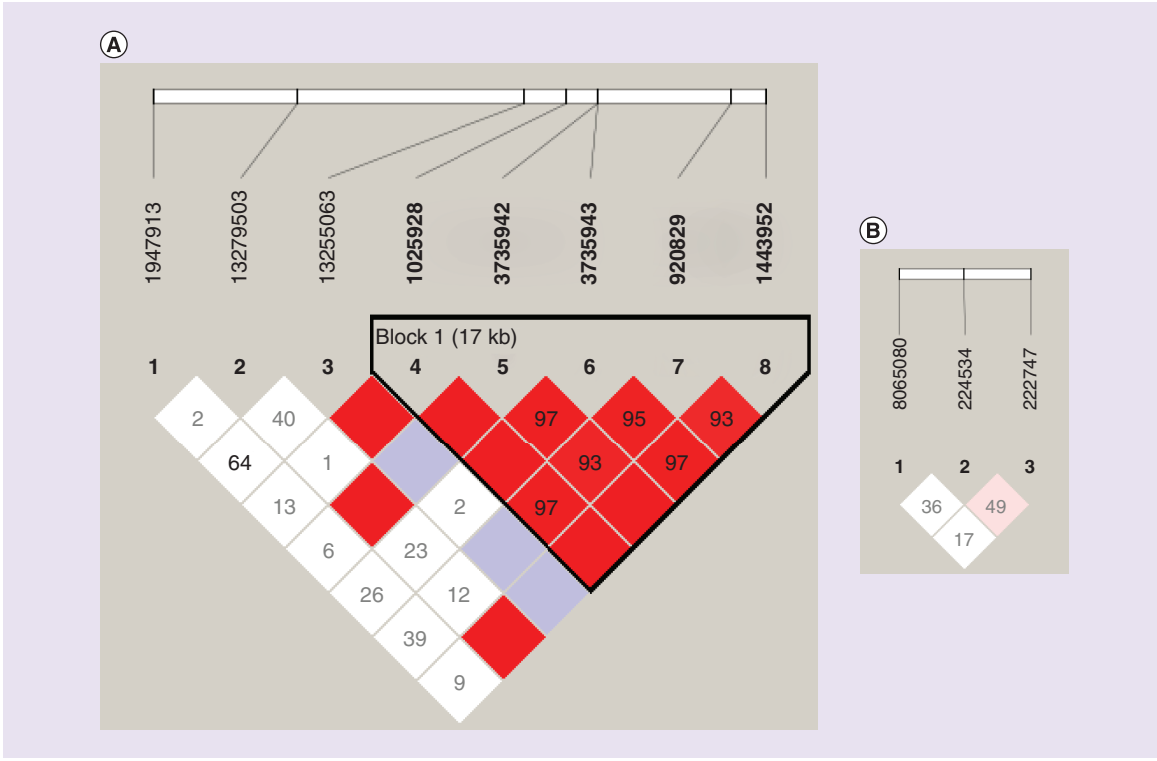

Figure 1. Linkage disequilibrium plot of *TRPV1* and *TRPA1* SNPs. Haplotype block organization of *TRPV1* and *TRPA1* SNPs generated from Haploview 4.2 using the standard D′/LOD color scheme. D′ values show the linkage disequilibrium coefficient. **(A)** LD plot of eight *TRPA1* SNPs. **(B)** LD plot of three *TRPV1* SNPs.
Blue: High D′ and low LOD; LOD: Logarithm of odds of two loci having linkage disequilibrium; Red: High D′ and high LOD; Shades of pink/red: Low D′ and high LOD. Haplotype blocks did not change when four subjects that were not self-reported African–American were excluded; White: Low D′ and low LOD.
LD: Linkage disequilibrium.
For color figures please see online at: https://www.futuremedicine.com/doi/full/10.2217/pgs-2017-0198

implicated in inflamed bladder or colon, bone cancer pain and after nerve injury or cutaneous inflammation [44,45]. TRPA1 bears 20% homology to TRPV1 on the peptide level and is coexpressed with TRPV1 in a subpopulation of unmyelinated nociceptive neurons. TRPA1 has been reported to mediate cold sensitivity in various neuropathic and inflammatory states [46–48], and its contributing functions in noxious cold transduction under naive conditions have been debated recently [12,14].

There are limited studies on functions of TRP channels in SCD pain. In a mouse model of severe SCD, SCD mice exhibited persistent hypersensitivity to mechanical, heat and cold stimuli. Mechanical sensitization of nociceptors at the terminal level in skin and at the membrane level in isolated somata was completely reversed, whereas mechanical allodynia was partially reversed by acute pharmacologic inhibition of the TRPV1 channel [17]. However, the roles of TRPV1 and TRPA1 in other pain phenotypes in SCD have not been examined in animal models or in clinical studies.

We have proposed a role of cold weather in precipitating pain in SCD patients with allodynic and hyperalgesic quality due to the development of neuropathic pain [49]. Combined with our observations in this study that *TRPA1* polymorphism and haplotype are associated with utilizations characterized by acute painful crisis in SCD patients, it might be explained by the contributing function of TRPA1 in cold pain transduction under neuropathic pain conditions. To further elucidate the results, we have in this study, a standardized quantitative sensory testing will certainly be useful to examine the association of cold sensation in SCD patients with different *TRPA1* polymorphism and haplotype.

There are limitations in our study. Although it would have been insightful to further analyze results according to all SCD types, we did not have a sufficient number of subjects with β^+ thalassemia or β^0 thalassemia to make this

approach meaningful. Findings from the current study may also be limited by the sample size; therefore, a large, multicenter, prospective study is needed to replicate the results.

To our knowledge, the current study is the first report to demonstrate an association between *TRPA1* gene polymorphisms and pain in SCD. The present data suggest that *TRPA1* rs920829 710G>A polymorphism and CGAGG haplotype are associated with an increased number of pain crisis in patients with SCD. Therefore, *TRPA1* polymorphisms may contribute to the heterogeneity of acute crisis pain in SCD.

Conclusion

TRPA1 polymorphisms may contribute to the heterogeneity of acute crisis pain in SCD. *TRPA1* rs920829 710G>A allele and CGAGG haplotype are associated with increased frequencies of acute crisis pain in patients with SCD.

Executive summary

- *TRPA1* rs920829 AA and AG genotypes showed a 68% increase in the rate of utilization over GG genotype.
- Each A allele of rs920829 contributed to a 44% increase in utilization rate.
- A significant association was found for *TRPA1* haplotype CGAGG (OR = 0.218, p = 0.009) with an increased rate of utilization.
- Our data suggest that *TRPA1* gene polymorphisms and haplotypes may contribute to the heterogeneity of acute pain in sicel cell disease.

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References

1 Platt OS, Thorington BD, Brambilla DJ *et al.* Pain in sickle cell disease. Rates and risk factors. *N. Engl. J. Med.* 325(1), 11–16 (1991).

2 Aisiku IP, Smith WR, McClish DK *et al.* Comparisons of high versus low emergency department utilizers in sickle cell disease. *Ann. Emerg. Med.* 53(5), 587–593 (2009).

3 Wilkie DJ, Molokie R, Boyd-Seal D *et al.* Patient-reported outcomes: descriptors of nociceptive and neuropathic pain and barriers to effective pain management in adult outpatients with sickle cell disease. *J. Natl Med. Assoc.* 102(1), 18–27 (2010).

4 Shankar SM, Arbogast PG, Mitchel E *et al.* Medical care utilization and mortality in sickle cell disease: a population-based study. *Am. J. Hematol.* 80(4), 262–270 (2005).

5 Smith WR, Scherer M. Sickle-cell pain: advances in epidemiology and etiology. *Hematol. Am. Soc. Hematol. Educ. Program* 2010, 409–415 (2010).

6 Ballas SK, Gupta K, Adams-Graves P. Sickle cell pain: a critical reappraisal. *Blood* 120(18), 3647–3656 (2012).

7 Brousseau DC, Owens PL, Mosso AL, Panepinto JA, Steiner CA. Acute care utilization and rehospitalizations for sickle cell disease. *JAMA* 303(13), 1288–1294 (2010).

8 Clapham DE. TRP channels as cellular sensors. *Nature* 426(6966), 517–524 (2003).

9 Caterina MJ, Schumacher MA, Tominaga M *et al.* The capsaicin receptor: a heat-activated ion channel in the pain pathway. *Nature* 389(6653), 816–824 (1997).

10 Jordt SE, Julius D. Molecular basis for species-specific sensitivity to 'hot' chili peppers. *Cell* 108(3), 421–430 (2002).

11 Chuang HH, Prescott ED, Kong H *et al.* Bradykinin and nerve growth factor release the capsaicin receptor from PtdIns(4,5)P2-mediated inhibition. *Nature* 411(6840), 957–962 (2001).

12 Story GM, Peier AM, Reeve AJ *et al.* ANKTM1, a TRP-like channel expressed in nociceptive neurons, is activated by cold temperatures. *Cell* 112(6), 819–829 (2003).

13 Tracey WD Jr, Wilson RI, Laurent G, Benzer S. Painless, a *Drosophila* gene essential for nociception. *Cell* 113(2), 261–273 (2003).

14 Kwan KY, Allchorne AJ, Vollrath MA *et al.* TRPA1 contributes to cold, mechanical, and chemical nociception but is not essential for hair-cell transduction. *Neuron* 50(2), 277–289 (2006).

15 Chen Y, Yang C, Wang ZJ. Proteinase-activated receptor 2 sensitizes transient receptor potential vanilloid 1, transient receptor potential vanilloid 4, and transient receptor potential ankyrin 1 in paclitaxel-induced neuropathic pain. *Neuroscience*193, 440–451 (2011).

16 Cortright DN, Szallasi A. TRP channels and pain. *Curr. Pharm. Des.* 15(15), 1736–1749 (2009).

17 Hillery CA, Kerstein PC, Vilceanu D *et al.* Transient receptor potential vanilloid 1 mediates pain in mice with severe sickle cell disease. *Blood* 118(12), 3376–3383 (2011).

18 Binder A, May D, Baron R *et al.* Transient receptor potential channel polymorphisms are associated with the somatosensory function in neuropathic pain patients. *PLoS ONE* 6(3), e17387 (2011).

19 Tahara T, Shibata T, Nakamura M *et al.* Homozygous TRPV1 315C influences the susceptibility to functional dyspepsia. *J. Clin. Gastroenterol.* 44(1), e1–e7 (2010).

20 Carreno O, Corominas R, Fernández-Morales J *et al.* SNP variants within the vanilloid *TRPV1* and *TRPV3* receptor genes are associated with migraine in the Spanish population. *Am J. Med. Genet. B Neuropsychiatr. Genet.* 159B(1), 94–103 (2011).

21 Ezenwa MO, Molokie RE, Wang ZJ *et al.* Outpatient pain predicts subsequent one-year acute health care utilization among adults with sickle cell disease. *J. Pain Symptom Manage.* 48(1), 65–74 (2014).

22 Melzack R. The McGill pain questionnaire: major properties and scoring methods. *Pain* 1, 277–299 (1975).

23 Wilkie DJ, Molokie RE, Suarez ML, Ezenwa MO, Wang ZJ. Composite pain index: reliability, validity, and sensitivity of a patient-reported outcome for research. *Pain Med.* 16(7), 1341–1348 (2015).

24 Ngamkham S, Holden JE, Wilkie DJ. Differences in pain location, intensity, and quality by pain pattern in outpatients with cancer. *Cancer Nurs.* 34(3), 228–237 (2011).

25 Wilkie DJ, Judge MK, Berry DL *et al.* Usability of a computerized PAINReportIt in the general public with pain and people with cancer pain. *J. Pain Symptom Manage.* 25(3), 213–224 (2003).

26 Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res.* 16(3), 1215 (1988).

27 Vandenbergh DJ, Anthony K, Whitfield KE. Optimizing DNA yield from buccal swabs in the elderly: attempts to promote buccal cell growth in culture. *Am. J. Hum. Biol.* 15(5), 637–642 (2003).

28 Gabriel S, Ziaugra L, Tabbaa D. SNP genotyping using the Sequenom MassARRAY iPLEX platform. *Curr. Protoc. Hum. Genet.* 2009 Chapter 2: (Unit 2), doi: 10.1002/0471142905.hg0212s60 (2009).

29 van Esch AA, Lamberts MP, te Morsche RH *et al.* Polymorphisms in gene encoding TRPV1-receptor involved in pain perception are unrelated to chronic pancreatitis. *BMC Gastroenterol.* 9, 97 (2009).

30 Nagar S, Remmel RP, Hebbel RP, Zimmerman CL. Metabolism of opioids is altered in liver microsomes of sickle cell transgenic mice. *Drug Metab. Dispos.* 32(1), 98–104 (2004).

31 Cameron A, Trivedi PK. *Regression Analysis of Count Data (1st Edition).* Cambridge University Press, Cambridge, UK (1998).

32 Lettre G, Lange C, Hirschhorn JN. Genetic model testing and statistical power in population-based association studies of quantitative traits. *Genet. Epidemiol.* 31(4), 358–362 (2007).

33 Clarke GM, Anderson CA, Pettersson FH *et al.* Basic statistical analysis in genetic case–control studies. *Nat. Protoc.* 6(2), 121–133 (2011).

34 Kloke JD, McKean JW. Rfit: Rank-based estimation for linear models. *R Journal* 4(2), 8 (2012).

35 Venables WN, Ripley BD. *Modern Applied Statistics with S.* Springer, NY, USA. (2002).

36 Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* 21(2), 263–265 (2005).

37 Purcell S, Neale B, Todd-Brown K *et al.* PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am. J. Hum. Genet.* 81(3), 559–575 (2007).

38 Purcell S (PLINK version 1.07). http://zzz.bwh.harvard.edu/plink/index.shtml

39 May D, Baastrup J, Nientit MR *et al.* Differential expression and functionality of TRPA1 protein genetic variants in conditions of thermal stimulation. *J. Biol. Chem.* 287(32), 27087–27094 (2012).

40 Kim H, Neubert JK, San Miguel A *et al.* Genetic influence on variability in human acute experimental pain sensitivity associated with gender, ethnicity and psychological temperament. *Pain* 109(3), 488–496 (2004).

41 Kim H, Mittal DP, Iadarola MJ, Dionne RA. Genetic predictors for acute experimental cold and heat pain sensitivity in humans. *J. Med. Genet.* 43(8), e40 (2006).

42 Tominaga M, Caterina MJ, Malmberg AB *et al.* The cloned capsaicin receptor integrates multiple pain-producing stimuli. *Neuron* 21(3), 531–543 (1998).

43 Caterina MJ, Leffler A, Malmberg AB *et al.* Impaired nociception and pain sensation in mice lacking the capsaicin receptor. *Science* 288(5464), 306–313 (2000).

44 Kanai Y, Nakazato E, Fujiuchi A, Hara T, Imai A. Involvement of an increased spinal TRPV1 sensitization through its up-regulation in mechanical allodynia of CCI rats. *Neuropharmacology* 49(7), 977–984 (2005).

45 McGaraughty S, Chu KL, Brown BS *et al.* Contributions of central and peripheral TRPV1 receptors to mechanically evoked and spontaneous firing of spinal neurons in inflamed rats. *J. Neurophysiol.* 100(6), 3158–3166 (2008).

46 da Costa DS, Meotti FC, Andrade EL *et al.* The involvement of the transient receptor potential A1 (TRPA1) in the maintenance of mechanical and cold hyperalgesia in persistent inflammation. *Pain* 148(3), 431–437 (2010).

47 del Camino D, Murphy S, Heiry M *et al.* TRPA1 contributes to cold hypersensitivity. *J. Neurosci.* 30(45), 15165–15174 (2010).

48 Obata K, Katsura H, Mizushima T *et al.* TRPA1 induced in sensory neurons contributes to cold hyperalgesia after inflammation and nerve injury. *J. Clin. Invest.* 115(9), 2393–2401 (2005).

49 Molokie RE, Wang ZJ, Wilkie DJ. Presence of neuropathic pain as an underlying mechanism for pain associated with cold weather in patients with sickle cell disease. *Med. Hypotheses* 77(4), 491–493 (2011).

50 Sherry ST, Ward MH, Kholodov M *et al.* dbSNP: the NCBI database of genetic variation. *Nucleic Acids Res.* 29(1), 308–311 (2001).